

Variation Pattern of mtDNA among Six Subspecies of *Apodemus agrarius* (Mammalia, Rodentia) in Korea, China, and Russia

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ABSTRACT

One hundred and eleven samples of six subspecies of striped field mouse, *Apodemus agrarius* Pallas from Korea, China and Russia, were used for the analysis of mitochondrial DNA (mtDNA) fragment patterns resulted from the digestion with eight restriction enzymes by blot hybridization technique. All 32 fragments, nine mtDNA haplotypes, and four major subgroups with the mean divergence value of 0.896 to 1.150% were revealed. In summary, three forms are recognized: [I, subspecies *chejuensis* (Chejudo island, Korea)], [II, subspecies *pallascens* (southwestern Korea), *coreae* (central Korea), and *septentrionalis* (Russia)], and [III, subspecies *manchuricus* (northeastern China) and *pallidior* (northern China)], although some samples of subspecies *coreae* are somewhat different from almost all samples of six subspecies, and some samples of subspecies *pallidior* are similar with all samples of subspecies *septentrionalis* to form same haplotype. It is confirmed that *A. agrarius chejuensis* is a distinct subspecies, that subspecies *coreae* (including *pallascens*) is also a distinct subspecies, that subspecies *manchuricus* and *pallidior* are synonyms of subspecies *ningpoensis*, and that subspecies *septentrionalis* is a synonym of subspecies *agrarius*. Moreover, it seems that *A. agrarius* shows constant karyotype, minimal variation in mtDNA genotype, and considerable divergence in morphometric characters, although further analyses with additional samples of *A. agrarius* in Eurasia will be necessary to determine the degree of variation of these taxonomic characters and to clarify subspecies classification as well.

Key words: Systematics, mtDNA, *Apodemus agrarius*, Korea, China, and Russia

INTRODUCTION

Striped field mouse, *Apodemus agrarius* Pallas, 1771, is widely distributed in Eurasia from Germany through Russia to Korea (Corbet, 1978). In Korea, Jones and Johnson (1965) reported four subspecies of *A. agrarius*: subspecies *pallidior* in the coastal lowlands of southern and southwestern Korea, subspecies *coreae* throughout the major portion of the peninsula, and subspecies *chejuensis* in Cheju island. In China, five subspecies of *A. agrarius* are recognized (Xia, 1984): *agrarius* from Omin, Tacheong, and northern Xinjiang; *ningpoensis*, middle and lower part of Yangtze Valley, Guizhou, and northern Fujian; *pallidior*, northern China, eastern part of northwestern China, Sichuan, and northern Jiangsu; *manchuricus*, northeastern China and eastern Inner Mongolia; *insulaemus*, Taiwan. In Russia, Ognev (1924) stated that in Russia, *A. a. septentrionalis* subsp. n. is smaller than subspecies *agrarius*, *ognevi*, and *manchuricus*.

The subspecies classification of *A. agrarius* is still in confusion (Kobayashi, 1985). Corbet (1978) summarized 24 nominal subspecies into three [*agrarius*, a western subspecies including 12 named subspecies from western Asia and Europe; *ningpoensis*, an eastern subspecies from eastern Asia including eight named subspecies; and *chevrieri*, a southern Chinese subspecies including one named subspecies]. Wang (1985) concluded that subspecies *chevrieri* is a distinct species. Koh (1986) concluded that *A. a. pallidior* is the synonym of *A. a. coreae* and Koh (1987) stated that *A. a. chejuensis* is distinct from *A. a. coreae*. Moreover, *A. a. coreae* of medium-size form is also different from other five subspecies of small-size form from China and Turkey, i.e., *manchuricus*, *pallidior*, *ningpoensis*, *insulaemus*, and *agrarius* (Koh, 1991).

Mitochondrial DNA studies are important to infer maternal lineages among closely related species and infraspecific populations (Avice, 1986), and the analysis of Restriction Fragment Length Polymorphisms (RFLPs) is a powerful and cost-effective method where large numbers of individuals or segments are being screened (Dowling *et al.*, 1990).

The purpose of this paper is to analyze the degree of differentiation in mtDNA restriction fragments among the samples of six subspecies of striped field mouse (*A. agrarius*) from Korea, China, and Russia (subspecies *chejuensis*, *coreae*, *pallidior*, *manchuricus*, *pallidior*, and *septentrionalis*) in order to reconsider their subspecific status.

MATERIALS AND METHODS

One hundred and eleven samples of six subspecies of striped field mouse (*Apodemus agrarius*), i.e., subspecies *chejuensis*, *pallidior*, and *coreae* from Korea, subspecies *manchuricus* and *pallidior* from China, and subspecies *septentrionalis* from Russia, were utilized (see Table 1 and Fig. 1).

Mitochondrial DNA was visualized from Southern blots (Southern, 1975) of total cellular DNA. DNA isolation from liver, electrophoresis, Southern transfer, and hybridization were described by Davis (1986). All samples were individually digested using the following eight restriction endonucleases: *Bam*H I, *Dra* I, *Eco*R I, *Eco*R V, *Hind* III, *Pst* I, *Pvu* II and *Stu* I. The mtDNA probe used

Table 1. Locality, number of samples, mitochondrial DNA genotypes, and haplotypes in six subspecies of striped field mice, *Apodemus agrarius*, from Korea, China, and Russia. Mitochondrial DNA genotypes are based on the fragment patterns resulted from the digestion with eight restriction endonucleases: *Bam*H I, *Dra* I, *Eco*R I, *Eco*R V, *Hind* III, *Pst* I, *Pvu* II, and *Stu* I, in order.

Subspecies	Locality (No. of samples)	OTU	mtDNA genotypes	Haplotypes
<i>chejuensis</i>	Cheju, Korea (8)	1	B A A A B A B A	6
<i>pallascens</i>	Kunsan, Korea (1)	2	A A A B B A A A	2
	" (5)	2	B A A B B A A A	8
<i>coreae</i>	Cheongju, Korea (2)	3	A A A B B A A A	2
	" (5)	3	B A A B A A A A	7
	" (14)	3	B A A B B A A A	8
	Mt. Songri, Korea (6)	4	A A A A B A A A	1
	" (3)	4	B A A A A A A A	3
	" (3)	4	B A A B B A A A	8
	Mt. Weolak, Korea (4)	5	B A A B B A A A	8
	Mt. Seolak, Korea (2)	6	B A A B B A A A	8
<i>manchuricus</i>	Changchun, China (6)	7	B A A A B A A B	5
<i>pallidior</i>	Beijing, China (8)	8	B A A A B A A B	5
	" (2)	8	B A B A B A A B	9
	Zibo, China (9)	9	B A A A B A A A	4
	" (1)	9	B A A A B A A B	5
	" (2)	9	B A B A B A A B	9
	Xiatung, China (7)	10	B A A A B A A B	5
	Mt. Tai, China (13)	11	B A A A B A A B	5
<i>septentrionalis</i>	Moscow, Russia (10)	12	B A A A B A A A	4

for all hybridization was pPld, which contains 10 Kb *Pst* I fragment of *Peromyscus leucopus* mtDNA genome in the vector pUc (courtesy of Dr. S. K. Davis in Texas A and M University).

In mtDNA analysis, each different restriction fragment produced by a particular enzyme is given a different (but arbitrary) letter. Sequence divergence in base substitutions per nucleotide (p in percent) was estimated from the restriction site data by the method of Upholt (1977). Phenogram was constructed from this data by Unweighted Pair Group Method with Arithmetic means, UPGMA (Sneath and Sokal, 1973).

RESULTS

A total of 32 fragments with eight restriction enzymes were recognized in 111 samples of six subspecies of *Apodemus agrarius* (see Table 1). The fragment patterns with *Dra* I and *Pst* I were not different among 111 samples of six subspecies of *A. agrarius* (OTUs 1, Korea, *chejuensis*;

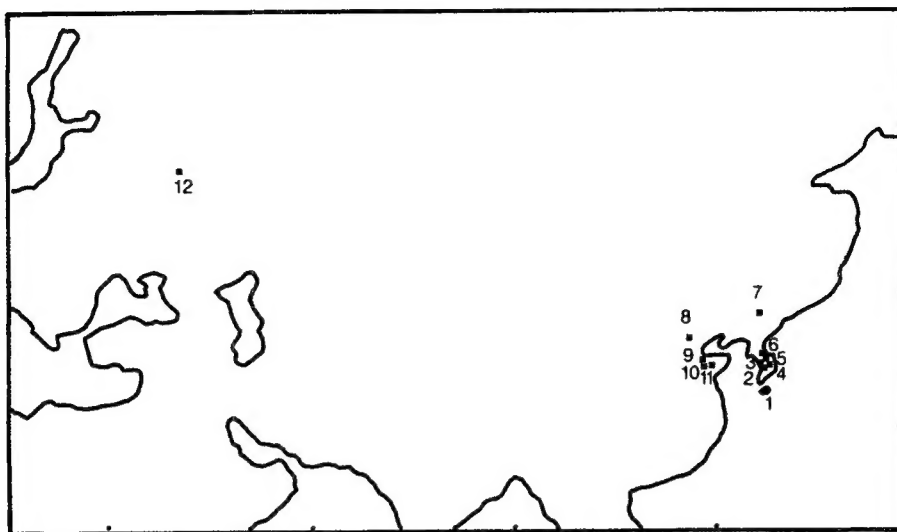


Fig. 1. Twelve localities of six subspecies of striped field mice, *Apodemus agrarius*, from Korea, China, and Russia. Alphabets indicate locality; Cheju island (1), Kunsan (2), Cheongju (3), Mt. Songri (4), Mt. Weolak (5), Mt. Seolak (6), Changchun (7), Beijing (8), Zibo (9), Mt. Tai (10), Xucheong (11), and Moscow (12). Subspecies name of samples in each locality refer to Table 1.

OTU 2, *pallidior*; OTUs 3, 4, 5, and 6, Korea, *coreae*; OTU 7, China, *manchuricus*; OTUs 8, 9, 10, and 11, China, *pallidior*; OTU 12, Russia, *septentrionalis*. However, type A with *Bam*H I were shown in one of six samples of subspecies *pallidior* and eight of 39 samples of *coreae*: type B in *Eco*R I were in four of 42 samples of subspecies *pallidior*: type B with *Eco*R V was in all six samples of subspecies *pallidior* and 30 of 39 samples of subspecies *coreae*: type A in *Hind* III was in eight of 39 samples of subspecies *coreae*: type B with *Pvu* II was found in all eight samples of subspecies *chejuensis*: and type B in *Stu* I in all six samples of subspecies *manchuricus*

Table 2. Nucleotide-sequence divergence among nine haplotypes in six subspecies of striped field mice, *Apodemus agrarius*, from Korea, China, and Russia. For locality and subspecies name of each haplotypes see Table 1.

Haplotype	1	2	3	4	5	6	7	8
2	0.406							
3	1.059	1.552						
4	0.141	0.829	0.566					
5	0.699	1.136	0.869	0.263				
6	1.136	1.136	1.004	0.697	0.697			
7	1.552	1.004	0.408	1.004	1.336	1.469		
8	0.829	0.412	1.004	0.412	0.697	0.817	0.556	
9	1.199	1.684	1.410	0.699	0.406	1.136	1.924	1.136

and 33 of 42 samples of subspecies *pallidior*.

Nine mitochondrial haplotypes were recognized in 111 samples of six subspecies of *Apodemus agrarius*, as shown in Table 1. Six samples of subspecies *coreae* in Korea are haplotype 1; one sample of subspecies *pallidior* and two samples of subspecies *coreae* from Korea are haplotype 2; three samples of subspecies *coreae* in Korea are haplotype 3; nine samples of subspecies *pallidior* from China and all ten samples of subspecies *septentrionalis* from Russia are haplotype 4; all six samples of subspecies *manchuricus* and 29 samples of subspecies *pallidior* in China are haplotype 5; all eight samples of subspecies *chejuensis* from Chejudo island from Korea are haplotype 6; five samples of subspecies *coreae* from Korea are haplotype 7; five samples of subspecies *pallidior* and 23 samples of subspecies *coreae* in Korea are haplotype 8; and four samples of subspecies *pallidior* from China are haplotype 9.

The nucleotide-sequence divergence (p in percent) among nine mtDNA haplotypes are given in Table 2. The grouping of nine haplotypes by UPGMA is shown in Fig. 2. Four major subgroups with the mean divergence value of 0.896 to 1.150% were revealed; [haplotypes 1, 2, 4, and 8, subspecies *pallidior*, *coreae*, *pallidior* (in part), and *septentrionalis*], [haplotypes 5 and 9, subspecies *manchuricus* and *pallidior*], [haplotype 6, subspecies *chejuensis*], and [haplotypes 3

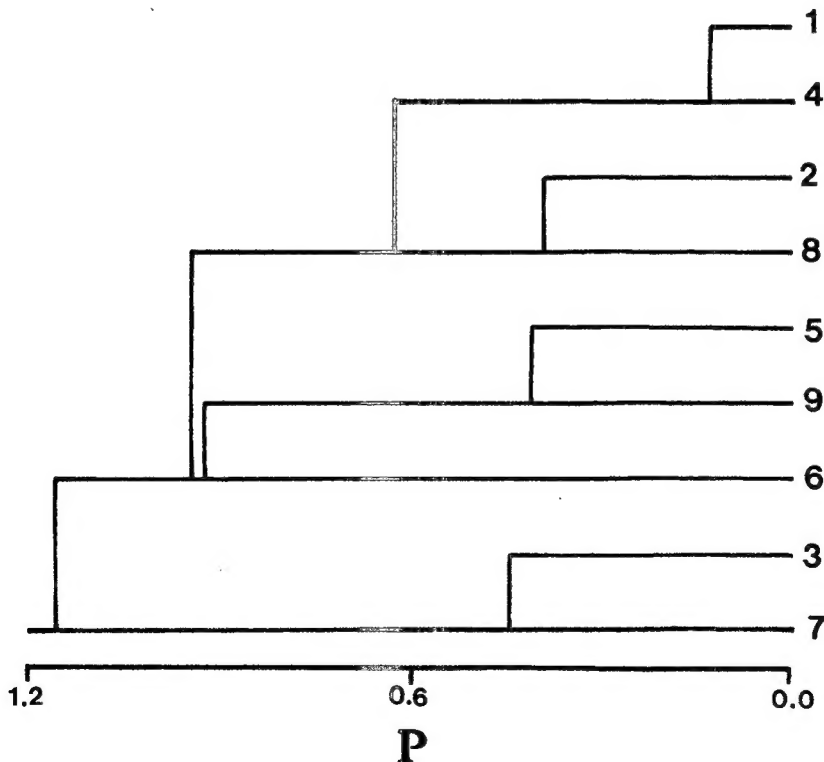


Fig. 2. Grouping of nine mitochondrial DNA haplotypes in six subspecies of striped field mice, *Apodemus agrarius*, from Korea, China, and Russia. The nucleotide-sequence divergences, given in Table 2, were used for UPGMA cluster analysis. Numerals refer to the haplotypes in Table 1.

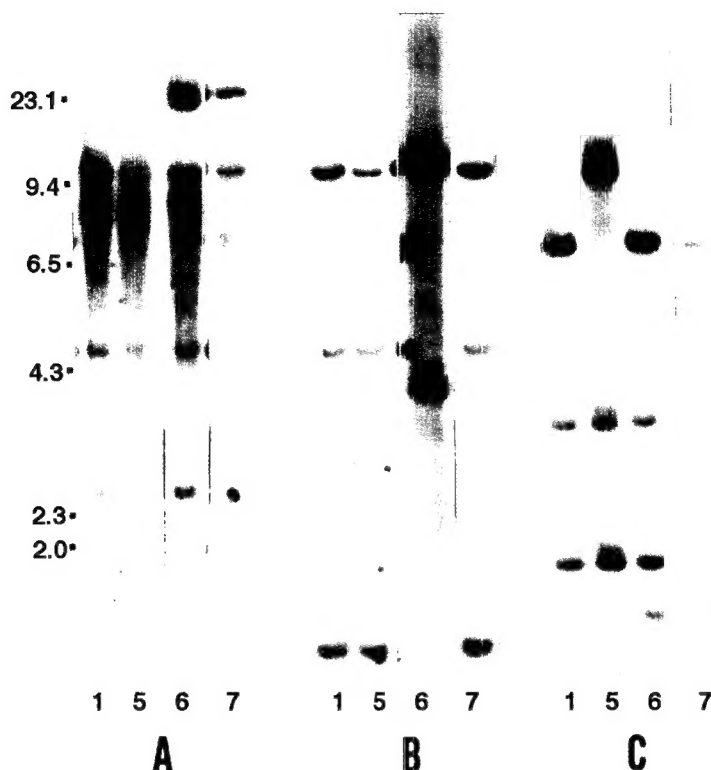


Fig. 3. Representative fragment patterns of six subspecies of striped field mice, *Apodemus agrarius*, from Korea, China, and Russia. Numerals indicate mtDNA haplotypes and the far left contains size markers indicated in kilo-base pairs. A, *Hind* III. B, *Pvu* II. C, *Stu* I. For subspecies name and locality of each haplotype see Table 1.

and 7, subspecies *coreae* (in part)].

In summary, based on mtDNA RFLP analysis with six subspecies of *Apodemus agrarius*, three forms are recognized: [I, subspecies *chejuensis* (Chejudo island, Korea)], [II, subspecies *pallidior* (southwestern Korea), *coreae* (central Korea), and *septentrionalis* (Russia)], and [III, subspecies *manchuricus* (northern China) and *pallidior* (central China)], although some samples of subspecies *coreae* are somewhat different from other samples of six subspecies, and some samples of subspecies *pallidior* is similar with all samples of subspecies *septentrionalis* to form the same haplotype.

Representative fragment patterns of mtDNA in haplotypes 1, 5, 6, and 7 with *Hind* III, *Pvu* II, and *Stu* I are shown in Fig. 3.

DISCUSSION

Sequence divergence of mtDNA estimated in *Rattus norvegicus* ranged from 0.2 to 0.8%

(Brown and Simpson, 1981). The maximum sequence divergence in *Clethrionomys rutilus* was 0.89% (Tegelstrom *et al.*, 1988). The mtDNA sequence divergence between Santa Catalina island and mainland harvest mice, *Reithrodontomys megalotis*, was less than 0.2% (Ashley, 1989). On the other hand, sequence divergence between *Peromyscus maniculatus* mtDNA clones from continental America was as high as 7% and seven assemblages were revealed (Lansmann *et al.*, 1983), and the divergence between eight mtDNA variants was 0.2 to 9.6% (Brown and Simpson, 1981). The greatest intraspecific sequence divergence among mtDNA clones of *Apodemus sylvaticus* was 1.4% and that of *A. flavicollis* was 1.0% (Tegelstrom and Jaarola, 1989). In this study with six subspecies of *Apodemus agrarius*, four major subgroups with the mean mtDNA divergence value of 0.896 to 1.150% were revealed (Fig. 2).

Developments in the areas of molecular, cyto-, and numerical taxonomy are enormous (Quicke, 1993), however, modern molecular techniques have not yet pushed comparative morphology into the shadows (Patterson, 1987), although de Jong (1998) noted that molecular studies might remodel the mammalian tree. It was advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Mayr and Ashlock, 1991; Huelsenbeck *et al.*, 1996). However, Wilson and Brown (1953) noted that different characters show independent trends of geographic variation, and it cause a problem on the designation of subspecies, and Omland (1997) stated that rates of molecular and morphological evolution may usually be coupled.

Corbet (1978) summarized 24 nominal subspecies of *A. agrarius* into three [*agrarius*, a western subspecies including 12 named subspecies from western Asia and Europe (*tianschanicus*, *ognevi*, *septentrionalis*, *nikolski*, *caucasicus*, *karelicus*, and *volgensis* and other five named subspecies of *albostratus*, *henrici*, *kahmanni*, *maculatus*, and *rubens*); *ningpoensis*, an eastern subspecies from eastern Asia including eight named subspecies (*coreae*, *pallidior*, and *chejuensis* in Korea, *manchuricus*, *pallidior*, and *insulaemus* in China, and other two named subspecies of *gloveri* and *harti*); and *chevrieri*, a southern Chinese subspecies including one named subspecies of *fergussoni*]. Kobayashi (1985) noted that it is necessary to reexamine the subspecies classification of striped field mouse, *A. agrarius*, which is widely distributed in Eurasia.

In the chromosomal studies on striped field mouse, *A. agrarius*, Kral (1970) reported that the karyotype of samples from Russia is diploid number of 48, i.e., 38 acrocentric autosomes, four pairs of small metacentric autosomes, large acrocentric X chromosome, and small acrocentric Y chromosome: the same karyotype was revealed in the samples from Korea (Koh, 1982) and those from China as well (Wang *et al.*, 1993), indicating that chromosomal karyotype might be constant in all subspecies of *Apodemus agrarius*.

In morphometric analyses of 15 subspecies of striped field mouse, *Apodemus agrarius* in Eurasia (Koh and Tikhonova, 1998), they concluded that 15 nominal subspecies can be classified into five subspecies: 1, a largest-size form, *chejuensis*, 2, a large-size form, *coreae* (including *pallidior*), 3, the other large-size form, *volgensis*, 4) a medium-size form, *ningpoensis* (including *manchuricus*, *pallidior*, and *insulaemus*), 5) a small-size form, *agrarius* (including *tianschanicus*, *ognevi*, *septentrionalis*, *nikolski*, *caucasicus*, and *karelicus*). In the analysis with mtDNA fragment patterns in *Apodemus agrarius*, subspecies *coreae* in mainland Korea appeared to be distinct from subspecies *chejuensis* from Cheju island, Korea (Koh and Yoo, 1992), and

subspecies *pallidior* from China appeared to be different from subspecies *coreae* from Korea (Wang and Koh, 1997).

In this study based on mtDNA RFLP analysis with six subspecies of *Apodemus agrarius* (Table 1 and Figs. 1 and 2), three forms are resulted: [I, subspecies *chejuensis* (Chejudo island, Korea)], [II, subspecies *pallidior* (southwestern Korea), *coreae* (central Korea), and *septrionalis* (Russia)], [III, subspecies *manchuricus* (northern China) and *pallidior* (central China)]. However, some samples of subspecies *coreae* are somewhat different from other samples of six subspecies: some samples of subspecies *pallidior* are similar with all samples of subspecies *septrionalis* to form same mtDNA haplotype, and they are more or less similar with subspecies *coreae* and *pallidior* to form same mtDNA subgroup.

Mayr (1969) noted that a subspecies is an aggregate of phenotypically similar populations of a species, differing taxonomically from other populations. The taxonomic status of six subspecies of *Apodemus agrarius* studied are confirmed such as followings. First of all, *A. a. chejuensis* is different from other 14 subspecies in its morphometric characters (Koh and Tikhonova, 1998), and it differs from other five subspecies in mtDNA genotypes (see Figs. 1 and 2), enough to be recognized as a subspecies. Johnson and Jones (1955) stated that *A. a. chejuensis* subsp. n. from Chejudo island is large, both externally and cranially, than any other described subspecies, but Corbet (1978) stated that the insular form from Chejudo island is rather large but is not very distinctive.

Secondly, *A. a. coreae* (including *pallidior*) is a distinct subspecies, which is different in their morphometric characters (Koh and Tikhonova, 1998) and mtDNA genotypes (see Figs. 1 and 2). Thomas (1908) stated that *A. a. coreae* subsp. n. has colour of cinnamon, which differs from subspecies *manchuricus* with more or less tawny colour, and Corbet (1978) regarded subspecies *coreae* as a synonym of subspecies *ningpoensis*. Moreover, the status of some samples, which has distinct mtDNA genotypes (see Fig. 2), is need to be analyzed in further studies, because geographic variation within species, hybridization, and cryptic species are all effectively studied with restriction site analysis (Hillis *et al.*, 1996).

Thirdly, *A. a. manchuricus* and *A. a. pallidior* are synonyms of *A. a. ningpoensis*, because three subspecies are similar in their morphometric characters (Koh and Tikhonova, 1998), and because subspecies *manchuricus* and *pallidior* are similar in their mtDNA genotypes with each other (see Figs. 1 and 2), although some samples of subspecies *pallidior* are similar with samples of subspecies *septrionalis*.

Thomas (1908) distinguished four subspecies of *A. agrarius* (subspecies *coreae*, *manchuricus*, *pallidior*, and *ningpoensis*) in China and Korea mainly based on dorsal stripe. Corbet (1978) stated that subspecies *ningpoensis*, an eastern subspecies from eastern Asia includes eight named subspecies (*coreae*, *pallidior*, and *chejuensis* in Korea, *manchuricus*, *pallidior*, and *insulaemus* in China, and other two named subspecies of *gloveri* and *harti*). However, in the comparative study of muscle proteins, Zhao and Lu (1986) analyzed samples in two subspecies of striped field mice from Shandong, Jiangsu, and Anhui provinces and concluded that *A. a. pallidior* from Shandong is distinct from *A. a. ningpoensis* from Jiangsu and Anhui provinces. Based on the comparison of black dorsal stripe and colour of their dorsal hair, Liu *et al.* (1991) noted that subspecies *pallidior* from northern part of its distribution is the synonym of subspecies *manchuricus*.

cus and that subspecies *pallidior* from southern part of its distribution is the synonym of subspecies *ningpoensis*. Further analysis with more samples of *A. agrarius* from China is necessary to clarify the taxonomic status of Chinese subspecies.

Finally, *A. a. septentrionalis* is a synonym of subspecies *agrarius* only because of their similarity in morphometric characters (Koh and Tikhonova, 1998), although subspecies *septentrionalis* has same mtDNA genotypes with some samples of subspecies *pallidior* and formed the same mtDNA subgroup with subspecies *coreae* (see Figs. 1 and 2). In Russia, Ognev (1924) compared morphometric characters of four subspecies of *A. agrarius*, and stated that subspecies *septentrionalis* is smaller than other three subspecies (*agrarius*, *ognevi*, and *manchuricus*). Corbet (1978) stated that *A. a. agrarius*, a western subspecies, includes 12 named subspecies from western Asia and Europe (*tianschanicus*, *ognevi*, *septentrionalis*, *nikolski*, *caucasicus*, *karelicus*, and *volgensis* and other five named subspecies of *albostratus*, *henrici*, *kahmanni*, *maculatus*, and *rubens*). Yonekawa *et al.* (1981) concluded that the mtDNA cleavage patterns does not provide support for all of the subspecies that have been described in *Mus musculus*. Mayr (1969) noted that taxonomists combine several more or less widely separated populations, which independently acquired an identical phenotype in a certain diagnostic character, into a single polytopic subspecies. But different characters show independent trends of geographic variation and several types of characters have to be considered before the designation of subspecies, as mentioned above.

Furthermore, it seems that *A. agrarius* shows constant chromosomal karyotypes, minimal variation in mtDNA genotypes, and considerable divergence of morphometric characters. In future, further studies with morphometric, chromosomal, and molecular characters of samples of all 23 nominal subspecies in *A. agrarius* will be necessary in order to confirm these conclusions.

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한국, 중국, 러시아에 서식하는 등줄쥐, *Apodemus agrarius*
(포유강, 설치목), 6아종의 미토콘드리아 DNA 절단단편의 변이

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요 약

한국, 중국, 러시아에 서식하는 등줄쥐 *Apodemus agrarius* 6아종 111마리를 이용하여, 미토콘드리아 DNA를 8개의 제한효소로 절단한 단편들을 blot hybridization법으로 분석하였다. 모두 32개의 단편들이 보여졌고, 9개의 haplotype이 밝혀졌으며, 평균 발산값이 0.896에서 1.150%인 4아군이 나타났다. 결론적으로 세 형을 인정할 수가 있었다: [I, 아종 *chejuensis* (제주도, 한국)], [II, 아종 *pallidior* (남서 한국), *coreae* (중앙 한국), 및 *septentrionalis* (러시아)], and [III, 아종 *manchuricus* (북동 중국)와 *pallidior* (북부 중국)]. 그러나 아종 *coreae*의 일부 표본들은 다른 6아종의 표본들과 다소 차이를 보였고, 아종 *pallidior*의 일부 표본들은 아종 *septentrionalis*의 모든 표본들과 같아서 동일한 haplotype을 형성하였다. 아종 *A. agrarius chejuensis*는 독특한 아종중의 하나이고, 아종 *coreae* (*pallidior*포함)도 독특한 아종중의 하나이며, 아종 *manchuricus*와 *pallidior*는 아종 *ningpoensis*의 동아종이명이고, 아종 *septentrionalis*도 아종 *agrarius*의 동아종이명임이 재확인되었다. 뿐만 아니라, 등줄쥐는 염색체 핵형이 일정하며, 미토콘드리아 DNA 유전형에 약간의 변이를 보이고, 형태형질이 상당한 발산을 보이고 있지만, 이들 분류형질의 변이 정도의 파악과 아종분류의 재검토를 위하여 유라시아산 등줄쥐의 보다 많은 표본을 이용한 분석이 앞으로 필요하다.